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14. ABSTRACT
We have designed an appropriate PET imaging protocol that will allow us to differentiate regional metabolic activity between different sleep cycle periods. Pilot diffusion MRI data using phantoms has informed a new and more accurate mathematical model, CFD-MRI, for MR tractography. The analysis indicates the necessity of further investigations for better understanding and quantifying various artifacts in data collection. Using the simultaneous fMRI-EEG data techniques, brain regions of interest and their activity has been identified. The results of these studies clearly indicate changes in resting state brain network connectivity in the transition from alert wakefulness to sleep.

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Neuroperformance, Sleep cycle, metabolism, thalamic structures, PET Imaging, MR Imaging, functional MRI, electroencephalography (EEG)

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Introduction

Sleep restriction and deprivation have profound negative effects on cognitive ability and task performance (e.g., vigilance). At the same time, physical and psychological stressors often lead to sleep disruptions, which compromise the body's ability to reap the restorative benefits of sleep. Military personnel often suffer from decreased **quality** and **quantity** of sleep, increasing their susceptibility to a host of neurological problems and limiting their ability to perform the challenging mental tasks that their missions require. Further, the effects of pharmacological interventions aimed at ameliorating the deleterious effects of both sleep loss and mental and physical stress are only poorly understood and may result in unanticipated long-term effects in those serving under combat conditions. Advances in imaging technology have enabled human studies of neurochemistry, energy metabolism and functional brain networks that were previously impossible.

Our research uses a combination of human imaging-based studies to advance our fundamental knowledge of the effects of sleep and sleep-related stressors on neuroperformance. In order to achieve this goal, the team utilizes ultra-high resolution imaging hardware, 7T MRI scanner and High Resolution PET (HRRT) scanner in tandem. These scanners at Neuroscience Research Institute at Gachon University are conveniently positioned so to be linked by a patient tray system, unique around the world, that can carry the subject from one scanner to the other, thereby keeping the reference frames of each modality intact and physically registered. The removal of software image registration from data analysis, as well as prevention of signal loss by the absence of physical/electrical interactions between the devices results in unprecedented high quality, ultra-high resolution images.

Adding this unique high precision setup to our capabilities, the scope of this research is to 1) use PET/MRI studies to understand glucose metabolism and dopamine binding in the brain, particularly in the brainstem and thalamus; 2) EEG/fMRI studies focusing on the functional connectivity of networks between the thalamus and cortex that control the descent into sleep and are altered by sleep deprivation; 3) EEG/fMRI studies designed to improve our understanding of the interaction between sleep loss, emotional stress and cognitive function, and 4) Development of reference image databases for high field MRI studies of the brain.

Body

The research accomplishments associated with each task outlined in the approved Statement of Work for year 1 are as follows:

A. PET/MRI studies of brainstem and cortex sleep regulation

A1. Provide sleep neuroscience expertise to the Neuroscience Research Institute (NRI) of Gachon University for PET/MRI studies.

A1.1. Training in the Neurobiology of Sleep and Sleep Medicine was provided by Dr. Larson-Prior. Since Fall 2012, NRI of Gachon University is capable of conducting experiments with no or minimal assistance from Dr. Larson-Prior.

A2. Analysis of pre-existing, de-identified PET/MRI data.

The analysis has not provided statistically significant results for distinguishing sleep stages between subjects based on brain metabolism measured using PET imaging data co-registered to high-resolution anatomical MRI data. Dr. Ozcan is looking into new approaches to see if further analysis could provide significant results.

Dr. Larson-Prior continues manuscript preparation on PET/MRI data from initial studies using FDG-PET in human subjects where the brainstem raphe nuclei were imaged in two major stages of normal sleep (SWS and REM) in normal healthy young adult subjects.

A3. Collaborative development of new data processing and analysis methods.
The task has been completed. However, the teams are still in collaboration for possible future development of analysis and data processing methods.

Dr. Ozcan has submitted the publication titled 'Complete Fourier Direct (CFD) NMR Resolves Crossing Fibers and Isotropic Component in a Biological Phantom' to the journal Magnetic Resonance in Medicine. It has been reviewed and Dr. Ozcan is preparing an updated version that will address the concerns of the reviewers in the second round.

A4. Recruit new subjects for studies of normal sleep using FDG and raclopride.
The task has been completed.

B. Studies of thalamo-cortical network function during sleep

B1. Analysis of pre-existing EEG/fMRI data on N1 and N2 sleep to determine changes in thalamo-cortical network connectivity in the descent to sleep.
Work on pursuing changes in thalamo-cortical connectivity further, using new methods for analyzing large-scale network activity continues with an outside collaboration with a biostatistician (Dr. William Shannon) with whom a novel approach for statistical analysis of these networks was developed. A manuscript describing that work is currently undergoing revision based upon reviewer's largely positive comments. The aim is to expand the original scope of this aspect of the project by functionally dividing the thalamic subnuclei and increasing the number of thalamic ROIs under investigation using a cortical parcellation scheme that does not assume *a priori* brain network memberships.

B2. Data collection and analysis to (1) increase the number of subjects reaching N2 sleep, and (2) collect data from subjects reaching N3 sleep.
Funding is not available to carry out this aim.

B3. Collect normative data on cognitive vigilance (PVT) prior to and following sleep (task flanks sleep) to analyze thalamo-cortical network interactions.

Studies are currently underway to investigate the role of sleep in the response of normal young adult subjects to combined emotional and psychological stress as proposed in the original application. These studies will use high-density (128 channel) electroencephalography (hdEEG) as the neuroimaging modality, with a subset of individuals (5 subjects) functional magnetic resonance imaging while performing the same task. The 5 subjects recruited to fMRI studies may also have participated in the hdEEG study and will provide information on the subcortical (e.g. thalamic) connectivity of the brain as subjects perform a demanding task under conditions of heightened psychological and emotional stress. The experimental paradigm for these studies is as proposed in the funded study.

Nineteen (19) healthy young adult subjects (ages 18-30, 10F) participated in a behavioral study designed to test the relationship between emotional stress and cognitive performance using a standard working memory task. Data analysis showed that subject response accuracy and reaction times, as expected, were significantly reduced under cognitive load (2-back working memory). While negative emotional interference conditions caused reductions in response accuracy and increases in reaction time, these effects did not reach statistical significance. We believe that this may have been an effect of relatively low subject numbers, but at present there are not funds available to investigate this further. Interestingly, the effect of negatively valenced emotional interference appears to dependent upon the degree to which subjects attended to the task at hand such that less interference was noted during the high cognitive load condition (2-back working memory) than in the low cognitive load condition (0-back working memory).

A sister project using the same task as that used in this study recruited 6 additional subjects to this behavioral task. These data were analyzed jointly with the following results:

- As previously noted, in the 0-back working memory task (low cognitive load), presentation of distractors that were negatively valenced (IAPS) significantly slowed reaction times (RT) while not affecting response accuracy relative to either no distractor, or a neutral (scrambled IAPS) distractor condition
- In the 2-back condition (increased cognitive load) there was no significant effect of negatively valenced distractors on RT but there was a significant effect on response accuracy.
- Correlational analyses investigating the role of sleep and mood in performance found that better overall emotional function (i.e. better quality sleep and good mood) resulted in a greater impact of negatively valenced emotional distractors (IAPS) on performance relative to conditions in which neutral emotional distractors or no distractors were presented.

Together, these results indicate that under non-demanding task conditions subjects pay more attention of distracting stimuli (non task-relevant), pulling their attentional focus away from the task at hand without reducing their performance accuracy. Alternatively, when the task demands are increased, subjects pay less overt attention to irrelevant stimuli while responding to increased emotional stress with reduced performance accuracy. Interestingly, subjects with poor sleep quality and greater self-reported depressive symptoms were less likely to show reduced performance in the face of negatively valenced emotional distractors.

A sister project in which this task was presented as subjects lay in a 3-tesla MRI scanner was performed in 5 healthy young adult subjects. Data has been collected in this study and is currently undergoing analysis. While these are pilot studies, it is hoped that funding can be obtained to further investigate the brain regions associated with the changes noted in our behavioral study.

During this final phase of the project, we will recruit subjects to an EEG study where they will perform the same task while have their brain activity recorded with scalp-recorded electrophysiology. Because our behavioral study resulted in very high performance levels (accuracy ranged from 80-99% on the 2-back task), further studies would both seek to increase cognitive load (include a 3-back working memory task) and increase task stress (add a PASAT mental serial subtraction task prior to the working memory task) to better assess the role of stress in cognitive performance. Based upon our behavioral results, we will require a minimum of 30 subjects to achieve statistically reliable results in this study. At present, funds are not available to begin recruitment for an EEG study.

C. Assemble and annotate a reference dataset of 7T brain images.

The brain atlas based on diffusion MRI data has been completed. The 7T magnetic resonance imaging diffusion data obtained at NRI has been post processed to correct for the susceptibility artifacts using the point spread function methodology. Subsequent to the corrections, track density imaging (TDI) calculations were executed for all anatomic orientations: axial, sagittal and coronal. For each anatomical orientation, three set of TDI images depicting the track densities in three anatomical orientations were calculated.
Using the TDI volume data, isosurfaces that show the brain structure were calculated. The surfaces were compactified to reduce the data size due to the large number of surface points. Both versions of the surfaces were saved in Virtual Reality Modeling Language (VRML) format. Full size files for each direction are approximately 1.15 gigabytes whereas the compactified file size is in the order of 0.5 gigabytes.
Accordingly, per orientation the full data size is ~1.65Gb which makes the total data size in the range of 14 (fourteen) Gbs.
The images were in all of the three anatomical orientations with the following specifications (in millimeters where appropriate):

	Axial	Coronal	Sagittal
Slice thickness:	2	2	2
Slice spacing	4	4	4

Pixel spacing	0.25\0.25	0.25\0.25	0.25\0.25
Image rows (height):	896	1024	1024
Image columns (width)	1024	896	896

The visualization of the three dimensional atlas is accomplished with open source, freely available software, Paraview. The software has been developed by Kitware (http://www.kitware.com/) and updates and downloads for various operating systems are available at the dedicated site: http://www.paraview.org/. Although a copy of the software is provided, it is highly recommended to obtain the latest version for taking full advantage of future developments.

Preparations in Anticipation of Future Data Collection and Analysis

The initial milestone of behavioral pilot testing has been completed, with 19 young adult subjects participating in this portion of the study.

Nineteen subjects (10F, ages 18-38) completed the behavioral pilot task, reporting finding the negatively valenced emotional distractors "disturbing" on a five-point Likert scale. On the 0-back working memory task without emotionally valenced distractors, subjects were highly accurate (0.98±0.04, mean ± SD), and accuracy decreased as expected with the increase in task difficulty in the 2-back task (0.96±0.03). Sleep quality, assessed by self-report using the Pittsburgh Sleep Quality Index (PSQI), ranged from excellent to mild difficulty (0-11). These data are currently being fully analyzed.

Five (5) subjects have participated in an fMRI scan in accordance with Year 1 scientific objectives under Dr. Larson-Prior's leadership at Washington University in St. Louis. However, as these studies were funded by the institution and not the DOD, these studies were undertaken using a sister protocol. Should funding be continued under the current project, new subjects will be recruited to the hdEEG portion of the year 1 study.

Scientific Workshop planned in June 2015

As a final action of this research project, a two-day scientific workshop is planned as below:

Working Title:	Role of Sleep in Cognitive Behavior and Human Performance
Date:	Early June 2015
Location	Greater Washington DC
Purpose:	Share the lesson learned interactively with interdisciplinary experts Design a scientific research roadmap for sleep research with new advanced tools
Scope:	15-20 scientific experts from DoD, VA, and Academia
Deliverable;	Publication in a scholarly journal

Key Research Accomplishments

- Completion of data collection and analysis of PET neuroimaging studies at Gachon University
- Completion of behavioral testing and analysis on the effects of sleep and emotional stress on neuroperformance
- Development of a novel new method for statistical analysis of connectomics data including manuscript submission (in revision)
- Submission of manuscript on improved analysis of DTI data (in revision)

Reportable Outcomes

Manuscripts,

- LaRosa, P., Brooks, T., Deych, E., Shands, B., Prior, F., Larson-Prior, L. and Shannon, W. *Gibb's distribution for statistical analysis of graphical data with a sample application to fcMRI brain images.* **Statistics in Medicine**, in revision.
- Alpay Ozcan, James D. Quirk, Yong Wang, Qing Wang, Peng Sun, William M. Spees, Seong K. Mun, Sheng-Kwei Song, Complete Fourier Direct (CFD) NMR Resolves Crossing Fibers and Isotropic Component in a Biological Phantom, first round of review completed at the journal Magnetic Resonance in Medicine.

Abstracts

- none

Presentations:

- none

Conclusion

Using simultaneous fMRI-EEG techniques, changes in large-scale network activity in the human brain have been identified based on resting state connectivity measures in the transition from alert wakefulness to sleep. An important change lay in the relationship between the default mode network and two networks involved in attending to and interacting with external stimuli. However, changes in subcortical-cortical connections have been less well defined despite a substantial body of data indicating that these changes do occur. While normal sleep is accompanied by clear substate-dependent changes in cortical network composition, no information is currently available on the changes that accompany abnormal sleep that has been affected by strong emotional and physical stress – conditions that are clearly present under battlefield conditions. In addition, the effects of these factors on waking cognitive performance are not fully understood and may be affected not only by age but also by personality characteristics. Dynamic changes in cognitive performance can only be measured by techniques that acquire data in the same time range as the brain computes such as magneto- and electrophysiology. Should additional funded become available, we will explore the effects of sleep quality on cognitive performance under emotional and cognitive stress. Behavioral data analyzed under this award have already resulted in new and interesting findings that would be enhanced by a greater understanding of the dynamic neural network interactions that underlies them. While the studies proposed here only mimic the stresses upon our warriors, they would provide valuable, even essential, information on the relationship between sleep need and neural stress that could inform not only optimal sleep durations and therapies under acute stress conditions, but therapeutic solutions to warfighters returning from the battlefield.

"So What" Section:

The outcomes of this Neuroperformance project, which is built on convergence of different disciplines, will have significant implications not only for warfighters but also the general population. Specifically our multi-modal approach, including structural information based on high resolution (7T MRI) anatomical images and diffusion MRI tractography (with information on white matter integrity) and functional information including metabolic activity (via PET), BOLD activity (via fMRI) and electrical activity (via EEG), are essential to neuro-biologically informed approach to improvements in neuroperformance under conditions of restricted sleep and high stress. In addition to improving our understanding of the effects of stress and sleep loss on neuroperformance, and to providing a firmer understanding of the effects of pharmacological interventions under these conditions, these studies will provide novel data on the multifactorial impact of sleep loss, stress and cognitive load in the development of neuropsychiatric disorders commonly associated with abnormalities of sleep such as depression and anxiety.

With information and knowledge accumulated in this project, further research areas which are in line with the goals and aims of the newly introduced BRAIN initiative would be developed. In fact, theoretical work has already been started for interpreting the fMRI data by adapting methodologies of the systems science and control theory. The collective interpretation of fMRI and EEG data using these mathematical approaches might become a Segway into the challenges posed by the BRAIN initiative including, producing a dynamic picture of the brain, demonstrating causality as well as advancing human neuroscience.

Appendices

NA

Abstracts:

Posters: